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Low Counts of Plasmacytoid Dendritic Cells after Engraftment Are Associated with High Early Mortality after Allogeneic Stem Cell Transplantation

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A B S T R A C T

Dendritic cells (DCs) are antigen-presenting cells that drive immune responses and tolerance and are divided in different subsets: myeloid DCs (mDCs: lineage−; HLA-DR+, 11c+), plasmacytoid dendritic cells (pDCs: HLA-DR+, CD123+), and monocyte-derived DCs (moDC: lineage−, 11c+, 16+). After hematopoietic stem cell transplantation (HSCT), low DC counts in the recipients' peripheral blood (PB) have been associated with worse outcomes, but the relevance of DC graft content remains unclear, and there are few data in the setting of unrelated donor HSCT. We evaluated the DC graft content and monitored DC recovery in PB from 111 HSCT recipients (median age, 17 years; range 1 to 74), who received bone marrow (46%), umbilical cord blood (32%), or PB (22%) from unrelated (81%) or related donors (19%). In 86 patients with sustained allogeneic recovery, patients with higher counts of all DC subsets (pDC, mDC, and moDC) 3 weeks after engraftment had lower incidence of nonrelapse mortality (NMR) and acute graft-versus-host disease (aGVHD) and better survival. pDC counts were associated with more striking results: patients with higher pDC counts had much lower incidences of NRM (3% versus 47%, $P < .0001$), lower incidence of aGVHD (24% versus 67%, $P < .0001$), and better overall survival (92% versus 45%, $P < .0001$). In contrast, higher pDC counts in the graft was associated with an increased risk of aGVHD (55% versus 26%, $P = .02$). Our results indicate that DC counts are closely correlated with HSCT outcomes and warrant further prospective evaluation and possible early therapeutic interventions to ameliorate severe aGVHD and decrease mortality.

INTRODUCTION

In recent years, hematopoietic stem cell transplantation (HSCT) has undergone significant improvement. However, the incidence of graft-versus-host disease (GVHD), infection, relapse, and mortality are still relatively high [1]. After transplantation, a cascade of complex and poorly understood events occur, which involves interactions of transferred and newly formed cells of donor origin with host cells. The type and intensity of these interactions will ultimately lead to different grades of acute GVHD (aGVHD), graft-versus-tumor (GVT) effects, and risk of infections, directly contributing to the success of the treatment [1].

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Dendritic cells (DC) are major antigen-presenting cells that act mainly through the activation or inhibition of T cells, leading to activation of Immune responses or tolerance [2-5], depending on their subtype and maturation status [6-8]. DCs represent a heterogeneous population of cells consisting of distinct subsets: myeloid (or conventional) DCs (mDC) and plasmacytoid DCs (pDC). Although mDCs exhibit proinflammatory responses leading to proliferation and activation of $TCD8^+$ cells to become cytotoxic, pDCs are involved on peripheral tolerance, viral host defense, and interferon production [2,3,9,10]. Circulating macrophages may also differentiate into DCs, being a potent allostimulatory cell subtype, referred to as monocyte-derived DCs, 16+/14− monocytes (moDC), or inflammatory DCs [11].

After HSCT, DC recovery in peripheral blood (PB) is relatively slow, and normal counts are only achieved after 3 months or later, depending on the recipients' clinical condition [12-14]. At such early phases (first 3 months), studies have shown that lower DC counts are associated with a higher presence of aGVHD and a poorer overall survival (OS), although their impact on disease relapse remains controversial [15-17]. Besides, some studies addressed the clinical relevance of DC content in the graft with conflicting results [15,18-20]. To date, only a few studies have simultaneously analyzed the pDC graft content, the kinetics of DC recovery, and the HSCT outcomes in the same patient population. In addition, most of these studies only included patients receiving grafts from matched related donors and determined DC counts in fixed time points, independent of day of engraftment or stem cell source.

In the present study, we analyzed DC recovery after transplantation and DC content in the graft in patients receiving allogeneic HSCT, mainly from unrelated donors. Using a novel strategy, considering the day of engraftment as baseline for DC recovery analyses, we investigated the association between the kinetics of DC recovery and the main HSCT outcomes.

PATIENTS AND METHODS

Patients from 4 transplantation centers were included. Each individual provided written informed consent to participate according to the Declaration of Helsinki. The study was approved by each center's local ethics committee.

PB samples were obtained at predefined time points as follows: before the conditioning regimen was administered, at engraftment, at days +3, +7, +14 and +21 after engraftment, and at days +60, +100 and +180 after transplantation. This strategy was used to normalize pDC recovery considering the engraftment date as the baseline in all patients, as engraftment is known to be delayed in umbilical cord blood (UCB) recipients compared with bone marrow (BM) and PB recipients. Graft samples were also analyzed when available (30 BM, 17 UCB, 13 PB).

Cells Populations Identification by Flow Cytometry

T CD4 lymphocytes, T CD8 lymphocytes, pDC (lineage−, HLA-DR high, CD123 high), mDCs (lineage−, CD11c+, CD16−), and moDCs (lineage−, CD11c+, CD16+) were quantified by 4-color flow cytometry. Briefly, fresh EDTA-anticoagulated PB or graft samples (mobilized PB, BM, or UCB) were stained with a combination of fluorochrome-conjugated monoclonal antibodies (CD3 APC, CD4 FITC, CD8 PE, CD11c APC, CD34 APC, CD45 Per-CP, CD123 PE HLA-DR FITC, [Becton Dickinson, San Jose, CA], CD16 PE [Immunotech] and Dendritic exclusion kit [Cytognos, Salamanca, Spain]). Samples were processed within 24 hours, and data were acquired for $\geq 10^5$ leukocytes/tube using the FACSCalibur flow cytometer (BD Biosciences). The Infinicity software (Cytognos) was used for data analysis. All analyses were performed in a central laboratory at UNIFESP by 2 independent investigators (Figure 1).

Statistical Analyses

For the analyses of the main clinical outcomes, patients were divided into 2 groups on the basis of different absolute cell count cutoffs for each time point. Using the Mann-Whitney test, we analyzed all quartiles of cell counts at each time point, and the median values of the absolute counts were used to separate 2 groups (high and low counts), as they showed the best correlation with the studied outcomes: aGVHD and chronic GVHD (cGVHD), diagnosed and graded according to published criteria [21,22], relapse or progression, nonrelapse-related mortality (NRM), progression-free survival (PFS), and OS.

Probabilities of PFS and OS were calculated using the Kaplan-Meier estimator and compared using the log-rank test, and cumulative incidence rates were calculated for aGVHD and cGVHD as well as relapse, with death being considered a competing event. Ninety-five percent confidence intervals (CIs) were estimated using the Greenwood formula. Adjusted probabilities for outcomes after transplantation were estimated using the Cox proportional hazards method. The association between cells counts and HSCT outcomes was investigated in the final multivariate models adjusting for patient-, disease-, and transplantation-related variables with an impact ($P < .01$) in the univariate analyses or if they had been reported to be clinically relevant. First-order interactions between DC counts and each variable of interest were examined. The results are presented as relative risks of failure (adverse prognostic factors versus good prognostic factors), with the 95% CIs and 2-sided P values. SPSS, version 20.0 (SPSS Inc., Chicago, IL), was used for all statistical analyses except for the cumulative incidence analyses, which were performed using S-PLUS software (TIBCO Software Inc., Palo Alto, CA).

RESULTS

Between May 2010 and November 2012, 111 patients (65% male; median age, 17 years; range, 1 to 74) from 4 transplantation centers were included. Chimerism data were evaluated during the first 3 months after HSCT. *Full donor chimerism* was defined as the presence of more than 95% of cells of donor origin. Patients who did not achieve neutrophil recovery or had neutrophil recovery but not full donor chimerism (n = 13), who died 1 week or earlier after engraftment (n = 10), or who were lost from follow-up (n = 2) were excluded from the analyses. There were no significant differences between the included and excluded patients groups regarding any of the clinical features (data not shown). Analyzed patient characteristics are presented in Table 1. Conditioning regimen, graft source, GVHD prophylaxis, time to transplantation, and all other clinical decisions were made according to each center's guidelines.

The most common underlying diagnosis was acute leukemia (70%). Patients received stem cells from BM (48%), UCB (29%), or PB (23%), derived from related (19%) or unrelated donors (81%). Most patients received a myeloablative conditioning regimen (62%), and one half of them (51%) received total body irradiation. T cell depletion with antithymocyte globulin was used in 37% of patients. Median follow-up was 24 months (range, 4 to 47).

Kinetics of DC Recovery

As expected, the median total nucleated cell (TNC) counts were almost 10-fold higher in BM and PB samples (4.43×10^8 TNC/kg) compared with UCB samples ($.56 \times 10^8$ TNC/kg). Similarly, DC counts were also approximately 10-fold higher in BM/PB, although the percentages were comparable among all stem cell sources (.19% in BM/PB versus .20% in UCB). However, no significant differences were noted regarding the kinetics of pDC, mDC, or moDC recovery after HSCT according to the type of stem cell sources used, except for slightly lower pDC counts at engraftment and at day +3 after engraftment and lower mDCs counts during the first 2 weeks in UCB recipients compared with BM/PB recipients. pDC and mDC counts remained relatively stable during the first 3 weeks after engraftment and started to increase at day +60 until day +100 in both patient groups, whereas the moDC count was stable and comparable to normal ranges very soon after engraftment, at day +3.

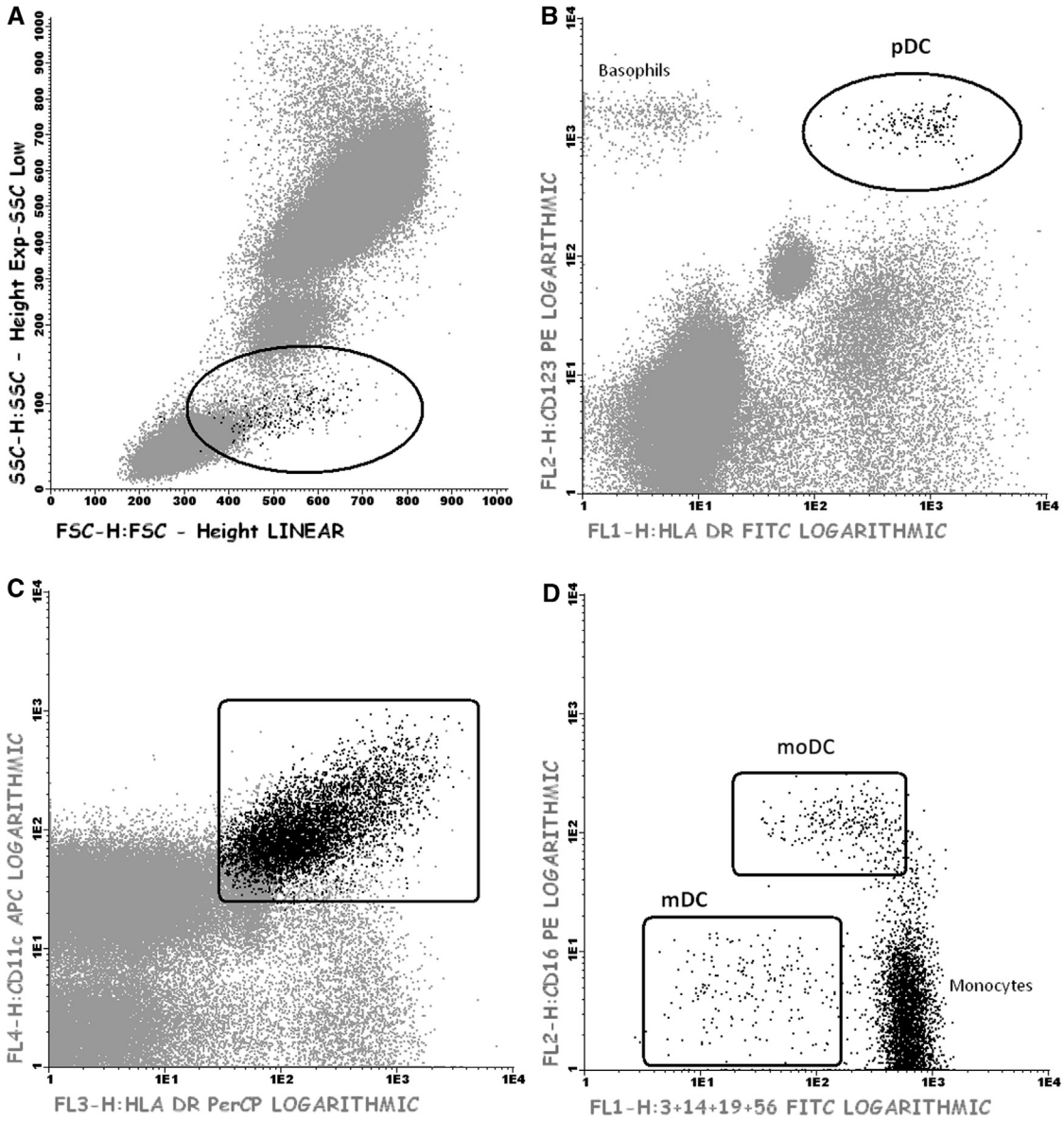

Figure 1. Flow cytometry identification of peripheral blood dendritic cells (DCs). As shown, plasmacytoid DCs (pDCs)(____) typically show low internal complexity (A) and high expression of both CD123 and HLA-DR (B). Basophils are shown as a positive internal control for CD123 staining, but they are HLA-DR negative. Myeloid DCs (mDC) and monocyte derived DCs (moDC) have high expression of HLA-DR and CD11c (C), are negative for lineage markers, and have distinct expression of CD16 (D).

DC recovery was not affected by any of the pre-transplantation characteristics that were investigated (ie, age, gender, conditioning regimen, use of total body irradiation or T cell depletion, and GVHD prophylaxis). Similarly, it was not affected by any of the host pre-HSCT subpopulations counts.

As seen in other studies on T cell recovery, BM and PB recipients presented a faster T cell recovery than UCB recipients, mainly due to fast elevation of T CD8, showing an early inversion of the CD4 to CD8 ratio.

DC Counts and NRM

Cumulative incidence of NRM was 30% at 1 year. Patients with lower DC counts after engraftment had a much higher risk of NRM than patients with higher DC counts (Table 2). The strongest association was noted for the pDC counts at day +21 after engraftment: NRM was 47% among the patients with lower counts (<1.1 cells/μL) and only 3% in patients with higher counts ($P < .0001$, Figure 2). Of note, in a multivariate analysis, pDC counts < 1.1 cells/μL at day +21 after engraftment was a powerful independent predictor of higher NRM (hazard ratio [HR], 16.67; 95% CI, 2.07 to 142.86) (Table 3) and the same was observed for pDC counts at day 7 and 14 after engraftment. In contrast, NRM was not affected by the pDC counts in the graft.

As for pDC, moDC and mDC counts on the first weeks after engraftment also showed significant correlation with NRM in univariate analyses. However in a multivariate analysis, mDC remained significant only at day 14 and moDC only at day 60 (Table 3).

DC Counts and GVHD

The cumulative incidence of grade II to IV aGVHD at day +100 was 43% with a median time to aGVHD of 31 days

Table 1
Patient and Disease Characteristics

Characteristic	Value
Patients, n	86
Age at transplantation, median (range), yr	18 (1-74)
Male sex	54 (63)
Diagnoses	
Acute myeloid leukemia	32 (37)
Acute lymphoid leukemia	28 (33)
MDS/MPN	13 (15)
Severe aplastic anemia	7 (8)
Others	6 (7)
Disease activity (malignant diseases, n = 75)	
Acute leukemia or lymphoma in complete remission, CML in chronic phase, MDS, MPN	60 (80)
Acute leukemia ≥ third remission or with active disease (relapse or progression)	15 (20)
Stem cell source	
BM	41 (48)
UCB	25 (29)
PB	20 (23)
Donor	
Unrelated	70 (81)
Related	16 (19)
Matched HLA (BM and PB)	
Matched	42 (69)
Mismatched	14 (23)
Haploidentical	5 (8)
Matched HLA (cord blood)	
Single	
No or 1 incompatibilities	7 (61)
Two incompatibilities	5 (39)
Double	
No or 1 incompatibilities per unit	3 (22)
At least 1 unit ≥ 2 incompatibilities	10 (78)
Reduced-intensity conditioning regimen	33 (38)
Total body irradiation	44 (51)
In vivo T cell depletion (antithymocyte globulin)	32 (37)
GVHD prophylaxis	
Cyclosporine or tacrolimus + MMF	36 (42)
Cyclosporine or tacrolimus + MTX	27 (31)
Cyclosporine or tacrolimus ± prednisone	15 (17)
Cyclosporine or tacrolimus + MMF+ post-transplantation cyclophosphamide	8 (9)
Follow up, median (range), mo	24 (4-47)

MDS indicates myelodysplastic syndrome; MPN, myeloproliferative neoplasm; CML, chronic myelogenous leukemia; MMF, mofetil mycophenolate; MTX, methotrexate.
Data presented are n (%) unless otherwise indicated.

(range, 10 to 100). Patients with lower pDC counts after engraftment had a much higher incidence of aGVHD than patients with higher pDC counts (Table 2), especially at days +21 and +60 after engraftment (67% versus 24% and 73% versus 30%, respectively) (Table 2, Figure 3). Multivariate analyses confirmed the independent predictive value of lower pDC counts at days 14, 21, and 60 after engraftment for higher risk aGVHD (day +21: HR, 3.31; 95% CI, 1.45 to 7.58) (Table 3).

Conversely, patients receiving a higher percentage of pDC in their grafts (≥.20% of TNC) had a significantly higher incidence of aGVHD at day +100 after HSCT than those with lower counts (55% versus 26%, $P = .02$) (Table 2) and this association remained significant in the multivariate analysis (HR, 2.98; 95% CI, 1.10 to 8.02) (Table 3).

Lower counts of mDC and moDC were also related to higher incidence of aGVHD, and this relation was confirmed in multivariate analyses at days 21 and 60 (Table 3).

A cumulative incidence of cGVHD of 52% was observed 1 year after engraftment. Similar to NRM and aGVHD, lower pDC counts at day +21 after engraftment was correlated with a higher risk of cGVHD: 83 versus 38% ($P = .008$) (Table 2). However, in a multivariate analysis, no independent predictive value of the pDC counts in cGVHD was observed. No association was found between occurrence of cGVHD and the pDC counts in the graft, mDC and moDC (Table 2).

DC Counts and Disease Relapse

Of the 86 evaluable patients, 76 had malignant underlying diseases, with a cumulative incidence of relapse or progression at 1 year of 22%. Neither pDC, moDC, mDC, nor T cells at any time point after transplantation had a significant impact on relapse or progression. The only significant factor that influenced relapse on multivariate analyses was advanced stage of the disease (HR, 5.19; 95% CI, 1.21 to 22.31).

DC Counts and Survival

OS was 62% at 1 year. During follow-up, there were 36 deaths: 10 related to disease relapse and 26 attributable to transplantation-related events. Eight died from aGVHD complications, 16 from infection without clinically relevant GVHD, 1 from cardiac arrhythmia, and 1 from secondary acute leukemia. Most patients died in the first 3 months after transplantation. Patients with higher pDC counts at day +21 after engraftment and at day +60 had a much higher probability of survival (Table 3): 92% versus 45% ($P < .0001$) (Table 2, Figure 4) and 93% versus 42% ($P = .001$) (Table 2), respectively. Lower pDC counts at day +21 and day +60 after engraftment remained a statistically independent predictive factor for higher OS in the multivariate analyses (at day +21: HR, 6.85; 95% CI, 1.92 to 24.39; Table 3; at day +60: HR, 6.43; 95% CI, 1.68 to 24.66). In contrast, OS was not affected by the pDC counts in the graft.

Also, mDC and moDC high counts positively correlated with OS. However, whereas moDC counts remained significant only at day +60 after multivariate analyses, higher mDC counts were associated with higher OS at days +14 (90% versus 44%, $P < .001$) (Table 3), day +21 (84% versus 52%, $P = .001$) (Table 3) and +60 (90% versus 58%, $P = .02$) (Table 3), remaining significant in a multivariate analysis (at day +21, HR: 4.31; 95% CI, 1.70 to 10.97; Table 3; at day +60: HR, 10.20; 95% CI, 2.24 to 46.48). T cells subpopulations had no impact on OS.

DISCUSSION

In this multicenter prospective study, we showed that low DC counts, especially pDC counts, after engraftment are strongly associated with poorer prognosis, reflected by higher NRM, greater incidence of acute GVHD, and shorter survival, in a cohort composed of patients receiving allogeneic transplantation, mostly from unrelated donors. The time point associated with the strongest association for all analyzed outcomes was day +21 (3 weeks after engraftment).

Our results are partially consistent with previously published series of patients receiving transplants mostly from related donors [15-17,23] and with a few small series of patients receiving transplants from unrelated donors [13,14,24-26]. In these studies, low DC counts after HSCT were associated with poorer survival, higher incidence of aGVHD, and higher rate of relapse, in some series. In our study, we did not find any association with DC counts and relapse. Therefore, the lower OS observed in patients with low DC counts reflects higher mortality from transplantation-related causes. All DCs had a significant association with outcomes, but pDCs were the subset with the most consistent association with both aGVHD and, more strikingly, NRM.

Table 2
Univariate Analyses of the Association of Dendritic Cell Counts and HSCT Outcomes

Outcome		NRM at 1 Year (%)				aGVHD at 100 Days (%)			cGVHD at 1 Year (%)			OS at 1 Year (%)		
Overall		30				43			52			62		
	n*	Cutoff† (cell/μL)	High	Low	*P* Value	High	Low	*P* Value	High	Low	*P* Value	High	Low	*P* Value
pDC 14	84	.91	16	33	NS	27	66	.04	52	40	NS	70	55	NS
pDC 21	84	1.1	3	47	<.0001	24	67	<.0001	38	71	.008	92	45	<.0001
pDC 60	73	1.7	4	37	.004	30	73	<.0001	43	65	.06	93	49	.001
mDC 14	84	3	3	45	.001	38	55	NS	40	57	NS	90	44	<.001
mDC 21	84	2.5	15	34	.04	27	62	.003	45	67	NS	84	52	.001
mDC 60	73	4.5	10	29	.04	31	70	.003	50	57	NS	90	58	.02
moDC 14	84	50	17	35	NS	30	63	.007	40	58	NS	73	53	NS
moDC 21	84	50	14	45	.04	30	60	.006	45	73	NS	83	45	.01
moDC 60	73	30	4	37	.005	37	63	.03	53	50	NS	96	50	<.001
pDC graft	60	.2% TNC	22	33	NS	55	2	.02	37	42	NS	58	57	NS

NS indicates not significant ($P > .05$).
Numbers 14, 21, and 60 after cell populations refer to day after engraftment. High and Low refer to patients with cell counts above or below the cutoff.
* n refers to number of patients available to analyze.
† Cutoff values measured in cells/μL, except for pDC graft content, measured in percentage of total TNC.

To the best of our knowledge, this was the first study in which the analysis of DC recovery was normalized at the day of engraftment, considering it baseline for all patients. All previously published studies [13-17,23-26] used fixed time points based on the day of transplantation (day 0), independently of stem cell source or $CD34^+$ cell dose. Based on the current knowledge of immune reconstitution kinetics, the use of a fixed time point, such as 1 month after HSCT, could lead to the comparison of patients at very different moments in the hematopoietic reconstitution phenomena, especially considering the inclusion of UCB transplant recipients that usually engraft later than recipients of other stem cell sources [27]. Besides, as we performed a weekly monitoring of DC counts, we could demonstrate the steps of DC reconstitution more closely than previously published series and determine that the first 2 and 3 weeks after engraftment were the most closely related to the outcomes.

DC counts were not affected by the pretransplantation patient characteristics (eg, age, conditioning regimen, T cell depletion, or GVHD prophylaxis) or by DC counts in the graft. The lack of correlation between DC counts in the graft and DC recovery in the PB after HSCT confirms previously published data [15,16,24]. We also demonstrated that despite receiving grafts with 10-fold fewer pDCs, once engraftment is established, UCB transplant recipients presented DC recovery rates that were comparable to the rates of those who received a transplant from other sources. Also, although the number of patients included does not allow subgroup analyses, multivariate analyses failed to demonstrated impact of graft source on outcomes. Based on these results, one could speculate that circulating DCs are newly formed and represent progeny of the donor stem cells rather than an oligoclonal expansion of transferred pDCs from the graft or host circulating DCs. Chimerism studies of DCs, and not only of the total mononucleated cells as performed in the present study, could help to clarify this aspect.

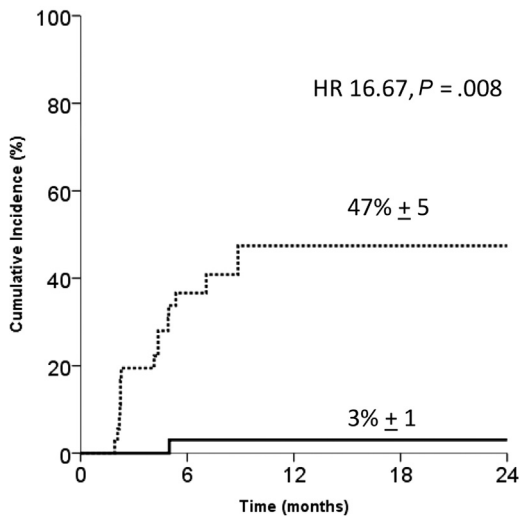

Figure 2. Cumulative incidence of nonrelapse–related mortality for patients with high (____) and low (.....) plasmacytoid dendritic cells counts (using a cutoff of 1 pDC/μL) measured 21 days after engraftment.

Whether a low number of DCs after engraftment is directly related to worse outcomes or if it is just a surrogate marker for inadequate immune recovery remains unknown. It remains to be determined whether pDC counts in the PB reflect lower pDC production or an increased pDC migration to tissues affected by infections and/or GVHD. Initial studies failed to demonstrate increased DCs in skin biopsies from patients with aGVHD [13,15,28], whereas in a more recent cohort, pDC were increased in the intestinal mucosa of patients with aGVHD [29]. Besides, as steroids have been shown to decrease the number of circulating pDCs, one might speculate that steroids used to treat aGVHD may also be the cause of lower DC counts after transplantation. However, in our series, as well as in other previous studies showing similar results, low pDC counts were reported several days before the onset of aGVHD in many patients and, therefore, before the onset of steroid therapy [12,30,31].

Indeed, the explanation for the strong power of DC counts in predicting outcomes after transplantation is still under debate. DCs are expected to play a central role in the immune reconstitution after transplantation. If there is an absolute decrease on total body host DCs, one could expect that patients would be more susceptible to infections because of ineffective antigen presentation and cytokine secretion. In fact, in our series, as well as in another series with 79 subjects, lower DC counts were associated with higher mortality due to infectious complications [16]. Indeed, activated DCs have strong antigen-presenting properties and are of extreme importance to protect against virus (pDC) and bacteria (mDCs and moDCs) proliferation, whereas steady-state

Table 3
Multivariate Analysis of Prognostic Factors for Main Outcomes after Transplantation

Outcome	*P* Value	HR	95% CI	
NRM				
<1.1 pDC/μL at day +21	.008	16.67	2.07	142.86
Cord blood transplantation	NS	3.24	.49	21.65
T cell depletion	NS	.42	.08	2.09
MMF prophylaxis	NS	.31	.05	2.03
<2.5 mDC/μL at day +14	.006	18.18	2.31	142.86
Cord blood transplantation	NS	1.22	.26	5.71
T cell depletion	NS	.57	.19	2.43
MMF prophylaxis	NS	.31	.06	1.51
<30 moDC/μL at day +60	.017	13.89	1.59	125
Cord blood transplantation	NS	3.59	.21	60.97
T cell depletion	NS	.10	.01	1.04
MMF prophylaxis	NS	.55	.03	9.04
aGVHD (at day +100 after transplantation)				
<1.1 pDC/μL at day +21	.005	3.31	1.45	7.58
Age >17 yr	.08	1.94	.91	4.11
T cell depletion	NS	.92	.39	2.18
MMF prophylaxis	NS	.68	.33	1.41
<3 mDC/μL at day +21	.016	2.67	1.20	5.92
Age >17 yr	.05	1.94	.99	4.42
T cell depletion	NS	.92	.35	1.93
MMF prophylaxis	NS	.68	.30	1.29
<50 moDC/μL at day +21	.03	2.28	1.09	4.83
Age >17 yr	.06	2.06	.97	4.32
T cell depletion	NS	.70	.31	1.60
MMF prophylaxis	NS	.66	.32	1.37
Graft pDC > .2% TNC	.01	2.98	1.10	8.02
UCB transplantation	.07	2.46	.93	6.54
T cell depletion	.08	.40	.15	1.11
Total body irradiation	.07	2.38	.94	6.04
cGVHD				
<1.1 pDC/μL at day +21	NS	2.15	.87	5.31
Age >17 yr	NS	1.72	.73	4.06
T cell depletion	NS	.53	.19	1.49
MMF prophylaxis	NS	.74	.32	1.75
OS (at 1 yr)				
<3.0 mDC/μL at day +21	.002	4.31	1.70	10.97
Cord blood transplantation	NS	.92	.25	3.37
T cell depletion	NS	.54	.19	1.56
MMF prophylaxis	NS	.48	.13	1.71
<1.1 pDC/μL at day +21	.003	6.85	1.92	24.39
Cord blood transplantation	NS	1.59	.37	6.87
T cell depletion	NS	.44	.12	1.59
MMF prophylaxis	NS	.54	.13	2.18
<30 moDC/μL at day +60	.002	28.57	3.39	250.0
Cord blood transplantation	NS	1.35	.21	8.56
T cell depletion	.018	.14	.03	.71
MMF prophylaxis	NS	1.67	.29	9.39

DCs have tolerogenic properties. Besides, a previous study showed that moDC, differently from monocytes, rapidly recovers immune properties after autologous transplantation and may contribute to infections control [32]. Only 1 study before our series analyzed moDC after allogeneic HSCT, having observed higher NRM, and higher relapse rates in patients with lower moDC counts [16].

Previous studies have analyzed the association of DCs with the complex pathophysiology of GVHD with controversial results: some studies in mice have shown that pDC or mDCs may initiate aGVHD whereas others have shown that pDC may protect against aGVHD [33-35]. In the clinical setting, all series, including ours, showed that a low DC counts in the PB after transplantation are consistently associated with a higher incidence of aGVHD both in adults and in children [13,15,16,24,36]. In our series, we observed that

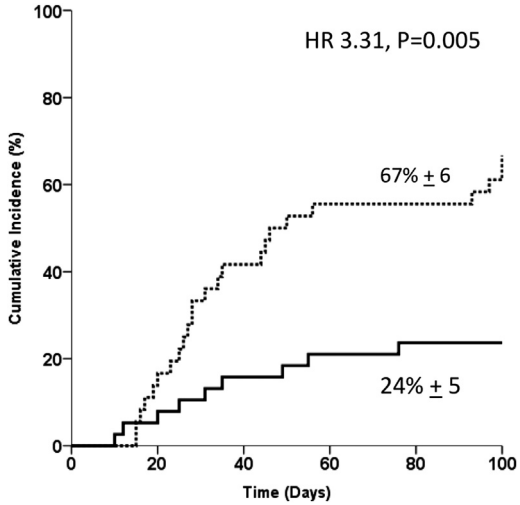

Figure 3. Cumulative incidence of acute GVHD for patients with high (_____) and low (.....) plasmacytoid dendritic cells counts at day +21 after engraftment.

pDC counts in the graft had an opposite effect on GVHD to that of pDC counts in the PB. Although this association was less significant, low pDC counts in the graft were associated with a slightly lower risk of aGVHD. Previously published series reported no impact of graft pDC counts on the incidence of aGVHD [15,18,20], although they did not include cases of UCB recipients and only a few cases of nonmyeloablative conditioning regimens. Studies with more homogeneous cohorts are needed to better elucidate the effect of DC graft content on clinical outcomes.

As a multicenter noninterventional HSCT study, our results, although very significant, should be interpreted with caution. HSCT procedures, conditioning regimens, and GVHD prophylaxis significantly differed between centers, as did the stem cell sources. However, despite the heterogeneity of the population included regarding age, disease, donor, stem cell source, HLA matching, conditioning regimen, and GVHD prophylaxis, none of the patient- or transplantation-related variables affected DC recovery. Besides, none of these

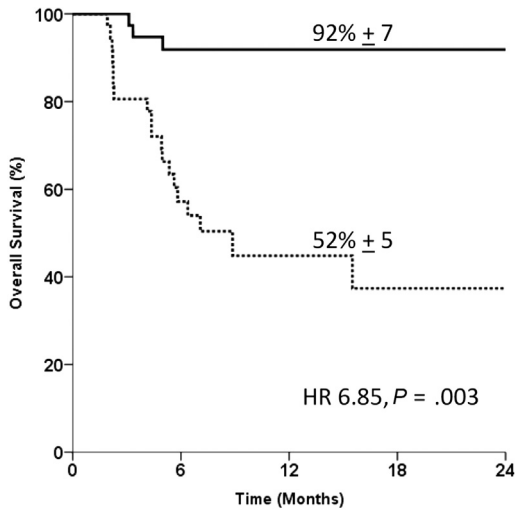

Figure 4. Overall survival for patients with high (_____) versus low (.....) plasmacytoid dendritic cells counts at day +21 after engraftment.

variables influenced the association between DC counts and patient outcomes, as confirmed in a multivariate analysis.

In summary, low DC counts, especially low pDC counts, observed in the first weeks after engraftment are associated with higher incidence of acute GVHD and mortality in allogeneic HSCT recipients. Our data support the critical relevance of DC recovery in the HSCT setting, independent of other clinical and biological variables. Determining DC counts after transplantation could further support the identification of patient at higher risk of GVHD and mortality and could warrant prospective evaluation and possible early therapeutic interventions. A better understanding of the role of DCs in the recovery of immunity after HSCT and the complex interactions of DCs with infective agents and host antigens requires further investigation.

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REFERENCES

1. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *NEJM*. 2010;363:2091-2101.
2. MacDonald KP, Munster DJ, Clark GJ, et al. Characterization of human blood dendritic cell subsets. *Blood*. 2002;100:4512-4520.
3. Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell*. 2001;106:259-262.
4. Darrasse-Jèze G, Deroubaix S, Mouquet H, et al. Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. *J Exp Med*. 2009; 206:1853-1862.
5. Luckashenak N, Schroeder S, Endt K, et al. Constitutive cross-presentation of tissue antigens by dendritic cells controls CD8+ T cell tolerance in vivo. *Immunity*. 2008;28:521-532.
6. Brilot F, Strowig T, Roberts SM, et al. NK cell survival mediated through the regulatory synapse with human DCs requires IL-15R alpha. *J Clin Invest*. 2007;117:3316-3329.
7. Borg C, Jalil A, Laderach D, et al. NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood*. 2004;104:3267-3275.
8. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol*. 2008;8:594-606.
9. Mittag D, Proietto AI, Loudovaris T, et al. Human dendritic cell subsets from spleen and blood are similar in phenotype and function but modified by donor health status. *J Immunol*. 2011;186:6207-6217.
10. Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med*. 2001;194:863-869.
11. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. *Trends Immunol*. 2013;34:440-445.
12. Fagnoni FF, Oliviero B, Giorgiani G, et al. Reconstitution dynamics of plasmacytoid and myeloid dendritic cell precursors after allogeneic myeloablative hematopoietic stem cell transplantation. *Blood*. 2004; 104:281-289.
13. Horváth R, Budinský V, Kayserová J, et al. Kinetics of dendritic cells reconstitution and costimulatory molecules expression after myeloablative allogeneic haematopoetic stem cell transplantation: Implications for the development of acute graft versus host disease. *Clin Immunol*. 2009;131:60-69.
14. Watanabe N, Narita M, Furukawa T, et al. Kinetics of pDCs, mDCs, gama delta T cells and regulatory T cells in association with graft versus host disease after hematopoietic. *Int J Lab Hem*. 2011;33:378-390.
15. Reddy V, Iturraspe JA, Tzolas AC, et al. Low dendritic cell count after allogeneic hematopoietic stem cell transplantation predicts relapse, death, and acute graft-versus-host disease. *Blood*. 2004;103:4330-4335.
16. Talarn C, Urbano-Ispizua A, Martino R, et al. Kinetics of recovery of dendritic cell subsets after reduced intensity conditioning allogeneic stem cell transplantation and clinical outcome. *Haematologica*. 2007; 92:1655-1663.
17. Rajasekar R, Mathews V, Lakshmi KM, et al. Plasmacytoid dendritic cell count on day 28 in hla-matched related allogeneic peripheral blood stem cell transplant predicts the incidence of acute and chronic GVHD. *Biol Bone Marrow Transplant*. 2008;14:344-350.
18. Waller EK, Rosenthal H, Jones TW, et al. Larger numbers of CD4(bright) dendritic cells in donor bone marrow are associated with increased relapse after allogeneic bone marrow transplantation. *Blood*. 2001;97: 2948-2956.
19. Rajasekar R, Lakshmi KM, George B, et al. Dendritic cell count in the graft predicts relapse in patients with hematologic malignancies undergoing an HLA-matched related allogeneic peripheral blood stem cell transplant. *Biol Blood Marrow Transplant*. 2010;16:854-860.
20. Waller EK, Logan BR, Harris WA, et al. Improved survival after transplantation of more donor plasmacytoid dendritic or naïve T cells from unrelated-donor marrow grafts: results from BMTCTN 0201. *J Clin Oncol*. 2014;32:2365-2372.
21. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
22. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
23. Lau J, Sartor M, Bradstock KF, et al. Activated circulating dendritic cells after hematopoietic stem cell transplantation predict acute graft-versus-host disease. *Transplantation*. 2007;83:839-846.
24. Porta MD, Rigolin GM, Alessandrino EP, et al. Dendritic cell recovery after allogeneic stem-cell transplantation in acute leukemia: correlations with clinical and transplant characteristics. *Eur J Haematol*. 2004; 72:18-25.
25. Kanda J, Chiou LW, Szabolcs P, et al. Immune recovery in adult patients after myeloablative dual umbilical cord blood, matched sibling, and matched unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2012;18:1664-1676.
26. Jacobson CA, Turki AT, McDonough SM, et al. Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. *Biol Bone Marrow Transplant*. 2012;18:565-574.
27. Rocha V, Gluckman E, Eurocord-Netcord Registry, European Blood and Marrow Transplant Group. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol*. 2009;147:262-274.
28. Auffermann-Gretzinger S, Eger L, Bornhäuser M, Schäkel K, et al. Fast appearance of donor dendritic cells in human skin: dynamics of skin and blood dendritic cells after allogeneic hematopoietic cell transplantation. *Transplantation*. 2006;81:866-873.
29. Bossard C, Malard F, Arbez J, et al. Plasmacytoid dendritic cells and Th17 immune response contribution in gastrointestinal acute graft-versus-host disease. *Leukemia*. 2012;26:1471-1474.
30. Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of Toll-like receptors. *Clin Immunol*. 2006; 120:260-271.
31. Arpinati M, Chirumbolo G, Urbini B, et al. Acute graft-versus-host disease and steroid treatment impair CD11c+ and CD123+ dendritic cell reconstitution after allogeneic peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2004;10:106-115.
32. Dayyani F, Joeinig A, Ziegler-Heitbrock L, et al. Autologous stem-cell transplantation restores the functional properties of CD14+CD16+ monocytes in patients with myeloma and lymphoma. *J Leukoc Biol*. 2004;75:207-213.
33. Koyama M, Hashimoto D, Aoyama K, et al. Plasmacytoid dendritic cells prime alloreactive T cells to mediate graft-versus-host disease as antigen-presenting cells. *Blood*. 2009;113:2088-2095.
34. Banovic T, Markey KA, Kuns RD, et al. Graft-versus-host disease prevents the maturation of plasmacytoid dendritic cells. *Biol Blood Marrow Transplant*. 2009;15:126-130.
35. Capitini CM, Nasholm NM, Chien CD, et al. Absence of STAT1 in donor-derived plasmacytoid dendritic cells results in increased STAT3 and attenuates murine GVHD. *Blood*. 2014;124:1976-1986.
36. Elze MC, Ciocarlie O, Heinze A, et al. Dendritic cell reconstitution is associated with relapse-free survival and acute GVHD severity in children after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2015;50:266-273.